AMENDMENTS TO THE DRAWINGS

Applicants concurrently submit herewith a new set of replacement formal drawings in black and white (Figures 1B; 2; 4; 5A-5B; 6; 7A-7F; 10A-10C; 11; and 12), in which the quality of the drawings have been improved in compliance with the requirement under 37 C.F.R. § 1.84.

Figure 1A is herein amended to delete the 323 base pairs at the 5'-end and the 33 base pairs at the 3'-end, all of which constitute the non-translated regions of the human BMP2 gene sequence, respectively, and were inadvertently included in Figure 1A (*i.e.*, SEQ ID NO:1). The nucleotide sequence of SEQ ID NO:1 encodes the amino acid sequence of SEQ ID NO:2 and, therefore, this amendment is supported by SEQ ID NO:2 and does not introduce any new matter. A marked-up version of Figure 1A is also attached hereto.

For the color photographs (*i.e.*, Figure 3A-3B; Figure 8A-8F; Figure 9A-9F; and Figure 13), a petition accompanied by three (3) sets of the color drawings and the required fee pursuant to 37 C.F.R. § 1.84(a)(2)(i) and (ii) is also submitted herewith. An amendment required under 37 C.F.R. § 1.84(a)(2)(iii) is included at page 3 of the present Amendment. No new matter has been introduced by this submission of the drawings.

AMENDMENTS TO THE SEQUENCE LISTING

In the original Sequence Listing, SEQ ID NO:1 inadvertently contained additional 323 base pairs at the 5'-end and 33 base pairs at the 3'-end, all of which constitute the non-translated regions of the human BMP2 gene sequence. Accordingly, Applicants submit herewith a Substitute Sequence Listing, in paper form and in computer readable form (CRF), in which the base pairs constituting the non-translated regions have been deleted. The nucleotide sequence of SEQ ID NO:1 encodes the amino acid sequence of SEQ ID NO:2 and, therefore, this amendment is supported by SEQ ID NO:2 and introduces no new matter.

"The Submission of Substitute Sequence Listing and Statement in Accordance with 37 C.F.R. §§ 1.821-1.825" is concurrently submitted herewith.

REMARKS

In the Office Action, the disclosure is objected to because of certain informalities. Drawings are objected to as not complying with 37 C.F.R. § 1.84. Claims 10-12, 13-16, 23-26 and 29-30 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The specification is herein amended to correct informalities and for clarification purposes. Support for the amendments to the specification can be found, for example, at page 4, lines 23-24; Figures 7 and 8; and at page 40, lines 17-32. A new set of drawings are concurrently submitted herewith. SEQ ID NO:1 is herein amended. A substitute Sequence Listing in paper form and in computer readable form is concurrently submitted herewith. Claims 1-9, 17-22, 27 and 28 are withdrawn as being non-elected. Claims 13, 15, 16, 23, 25, 29 and 30 are herein amended. Support for the amendments can be found, for example, at page 4, lines 21-24 and lines 29-31; at page 7, lines 7-16; at page 14, lines 22-27; at page 41, lines 7-10; and Figure 8. Claims 10-12 are herein cancelled without prejudice. New claims 31-48 are herein added. Support for new claims can be additionally found, for example, at page 24, lines 21-29; at page 25, lines 6-10 and at page 41, lines 4-6. Claims 13-16, 23-26 and 29-48 are pending in the case. No new matter has been introduced by the amendments.

Reconsideration of the present application in view of the foregoing amendments and the remarks below is respectfully requested.

Claim Rejections under 35 U.S.C. § 112

Claims 10-12, 13-16, 23-26, 29 and 30 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement.

Specifically, the Office Action states that the claims contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use

the invention and cites the eight factors set forth in *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988).

Claims 10-12 are herein cancelled without prejudice. Accordingly, the rejection of claims 10-12 are now moot.

Claims 13 and 23 are herein amended to become directed specifically to a method for treating a disease or disorder in a body area of an immunocompetent subject ***where bone regeneration is required***, by ***locally*** administering the nucleic acid molecule of the invention.

Claim 29 is herein amended to become directed to a method for ***treating a diseased or injured body area*** of an immunocompetent subject by ***locally*** administering the nucleic acid molecule of the invention comprising a nucleotide sequence encoding a therapeutic gene product.

The present invention as disclosed in the specification is discussed below in view of each factor set forth in *In Wands*:

The Breadth of the Claims

In the present invention, the inventors have demonstrated the synergistic effect of combining AAV and adenovirus for ***in vivo*** gene therapy. In particular, the most remarkable breakthrough regarding the present invention is that by combining a therapeutically effective amount of AAV-BMP2 and a sub-therapeutic dose of Adv-BMP2, a significant enhancement of new bone formation by approximately 10-fold was achieved (*see* Sections 7.4 and 7.5 at page 39 through 40 of the present specification).

Previously, Goncalves et al. (2001, *Virology* 288:236-246) disclosed a hybrid vector of AAV/Adenovirus to realize high transduction efficiency. Ferrari et al. (1996, "Second-strand synthesis is a rate-limiting step for efficient transduction by recombinant adno-associated virus vectors", *J. Virol.* 70:3227-3234; submitted previously as CD2 in the Information Disclosure Statement) further showed that the in vitro transduction

efficiency of recombinant vector of AAV carrying the lacZ gene was largely enhanced by the presence of adenovirus. However, none of the prior art references discloses the synergistic effect of combining AAV and adenovirus, both carrying the therapeutic gene, for ***in vivo*** gene therapy.

The present inventors have not only discovered the synergistic effect produced by the cocktail gene delivery system combining AAV and adenovirus, but also been successful in designing said system in such a way that no adverse immune response will be elicited by the presence of adenovirus in the treated subject. Specifically, in the cocktail vector system of the present invention, the adenovirus vector is present at an experimentally non-cytotoxic dosage, which is approximately 100- to 1000-fold lower than that of AAV vector (*see* page 15, lines 1-3; and Section 7.5, of the present specification), so that no adverse immune response will be elicited in the treated subjects.

The present inventors have successfully demonstrated that the cocktail vector system of the present invention can efficiently express a BMP at the site of the administration in a subject, thereby effecting the bone regeneration at the site. Furthermore, this system is applicable to expressing various other polypeptides/proteins having therapeutic effects at a targeted site in a subject by locally administering the cocktail of AAV and adenoviral vectors carrying a nucleic acid molecule encoding such polypeptides. Thus, the present invention also encompasses a method of treating a diseased or injured body area in a subject using the cocktail vectors carrying a nucleic acid molecule encoding a therapeutic gene product.

The Amount of Direction and Guidance Provided by the Specification

The present specification discloses the AAV-BMP2 and Adv-BMP2 cocktail vector system that can be used for treating any disease where bone regeneration is required, in immunocompetent subjects including humans. In *in vivo* Sprague-Dawley rat model, the present invention demonstrated a markedly enhanced new bone

formation by injecting AAV-BMP2 (5×10^{11} viral particle or v.p.) intramuscularly with a minute amount of Adv-BMP2 (5×10^{8} v.p.). The dosages and administration route disclosed in the present specification are easily adjustable and applicable to expressing and locally treating other types of diseases and disorders as disclosed in Sections 5.3 and 5.4. Thus, the present specification provides sufficient direction and guidance for practicing the present invention as recited in the amended claims.

The Existence of Working Examples

Examples 7.4 and 7.5 actually demonstrated the formation of new bone, but not bone-like structure, around the areas injected with AAV-BMP2 (5×10^{11} v.p.) plus Adv-BMP2 (5×10^{8} v.p.). This finding has been confirmed by both X-ray radiography and hematoxylin and eosin (H&E) staining. Furthermore, immunostaining using the human BMP2 antibody showed that strong BMP2 protein expression is co-localized with the tissue trabeuclar bone matrix. Therefore, it is predictable that at the above stated dosages, injection of the AAV-BMP2/Adv-BMP2 cocktail vector into the skeletal muscle can induce new bone formation at least in the immunocompetent rats.

The Nature of the Invention

Despite the challenges faced by the current gene therapy strategies, which have been pointed out by the Examiner, gene therapy still provides new hopes for terminal or incurable diseases where conventional therapies appear to be ineffective. Recently, Gendicine, or recombinant adenovirus engineered to express the p53 tumor suppressor gene (rAdv-p53), has been approved as a gene therapy product by the State Food and Drug Administration of China (SFDA) for the treatment of patients with head and neck squamous cell carcinoma (by Z. Peng, 2005, *Human Gene Ther.*, 16:1016-1027).

For *in vivo* gene therapy, recombinant AAV vectors of various serotypes have also become the method of choice for therapeutic gene transfer because of their high

biosafety profiles as compared to recombinant adenoviruses. In particular, recombinant AAV serotype 2 (rAAV2) vector has gained popularity because of its lack of pathogenicity and ability to establish long-term transgene expression. Such advantages of AAV vectors were well known at the time of the filing of the present application and is also discussed, for example, at page 2, lines 18-30, of the present specification. Currently, rAAV2 vectors have been examined in preclinical studies for the treatment of many human diseases, including hemophilia, alpha-1 anti-trypsin deficiency, cystic fibrosis, Duchenne muscular dystrophy, and rheumatoid arthritis.

However, there are several disadvantages associated with AAV vectors. The cost of producing high titer AAV free of Adv contaminations is relatively high, while the efficacy for gene delivery and expression is relatively low. This is in contrast to the adenoviral vectors which are highly efficient and capable of infecting a significant proportion of cells. However, adenovirus is associated with immune rejection, cellular toxicity and inflammatory reactions, which has limited its uses. There has been no prior art which could solve the disadvantages presented by each of the vectors until the present invention.

The present invention of a combined Adeno Associated Virus (AAV) plus Adenovirus (Adv) cocktail gene delivery system achieves a level of gene expression approximately 10 fold higher than that of AAV alone (*see* Sections 7.4 and 7.5 at page 39 through 40 of the present specification). This is accomplished without eliciting the undesirable immune responses seen in the adenovirus-based vectors *in vivo* in immunocompetent animals.

Thus, the combined AAV and Adv cocktail gene therapy of the present invention offers the following advantages:

1. More cost effective than AAV gene therapy. High titer of AAV free of Adv is expensive and difficult to produce. Based on the present results, the cost of therapy can be reduced by at least two mechanisms. Firstly, there is no need to remove the

entire Adv virus as long as the Adv level is within the immuno-tolerent level (e.g. ≤ 5 x 10^8 particles of Adv in rat muscle). Therefore, the more cost effective wild-type Adv-dependent AAV producer cell lines can be used. Secondly, a reduced level of AAV can be used to achieve optimal therapeutic results.

2. Higher efficacy as compared to AAV single vector gene therapy. For example, the present inventors have demonstrated that a reduced AAV plus minute dose of Adv combinational gene therapy produces approximately 50% higher efficacy than that of the high titer AAV therapy in the delivery of BMP2 gene product in rat muscle (*see* Sections 7.4 and 7.5).

3. No detectable immune responses as compared to the single Adv vector system. Therefore, the AAV and Adv cocktail vector system can be used as a safe alternative to the more commonly used Adv vector system.

It is predictable that the present AAV-BMP2/Adv-BMP2 cocktail vectors can also achieve long-term BMP2 expression in the transduced muscle cells for promoting the new bone formation and repair processes in immunocompetent subjects. Thus it is also predictable that in vivo administration of an AAV plus Adv cocktail vector system comprising other therapeutic genes can achieve long-term gene expression of the therapeutic gene products for the treatment of diseases.

The State of the Prior Art

The Examiner points out that the prior art for gene therapy involving BMPs often relates to bone regeneration, but not to any particular disorder or disease (p. 13, first paragraph). Further, the Examiner cites Alden et al. (1999) *J. Neurosurg. Spine* 1, 90:109-14, and goes on to state that it is not predictable that the concentrations made in the transduced skeletal muscle can induce bone formation at a side ***distant to the site of injection*** (p.14, 2nd paragraph).

However, the present inventors have demonstrated that the enhanced expression of BMP2 protein in the transduced muscle cells can be achieved by ***locally*** administering a sufficiently high dosage of the cocktail vectors as disclosed in the present specification and shown remarkable induction of new bone formation ***locally around the injection site*** (*see* Figures 10A-10C). Guided by the X-ray radiography, it is not difficult for any Artisan to inject the AAV-BMP2/Adv-BMP2 vectors directly to the muscle adjacent to where a bone fracture or fracture non-union occurs. It is predictable that when the injected muscle lies in close proximity to the fracture area, the chance that the newly formed bone can fuse to the endogenous bone should be reasonably high.

Likewise, when the cocktail vectors in which the BMP-encoding nucleic acid molecule is replaced by a nucleic acid molecule encoding other therapeutic polypeptides and administered locally at an afflicted body area of a subject, it is reasonably expected that such polypeptides would be expressed and exhibit their therapeutic effects at the site of administration.

The Level of Ordinary Skill and Predictability in the Art

There was a high level of skill in the field of DNA engineering and gene therapy at the time when the present application was filed. Despite a certain degree of unpredictability in the field of gene therapy, the present invention have provided solid evidence showing that the combination of a therapeutically effective amount of AAV-BMP2 (5×10^{11} v.p.) and a minute sub-therapeutic amount of Adv-BMP2 (5×10^{8} v.p.), when delivered intramuscularly, can significantly enhance new bone formation in immunocompetent rats at the site of injection. For inducing bone regeneration in larger immunocompetent subjects like humans, the amount of the cocktail vectors can be scaled up accordingly.

Thus, the present disclosure has provided reasonable predictability that a locally administered cocktail vectors of the present invention carrying a nucleotide

sequence, which encodes other therapeutic polypeptide, can optimally express such a polypeptide locally at the site of administration. Furthermore, given a high level of skill in the field of DNA engineering, one skilled in the art would have been able to modify the cocktail vectors disclosed in the present application so as to replace the nucleotide sequence of BMP with a nucleotide sequence encoding other materially different gene product for treating other human diseases, such as cancer. Examples of such gene products are listed at page 24, line 30 through page 25, line 4 and at page 25, lines 13-16.

The Amount of Experimentation Required to Practice the Invention

The present disclosure provides the following important information for practicing the invention:

1. **Scope of treatment:** The AAV/Adv cocktail vectors are used for approximately 10-fold enhancement of the expression of therapeutic gene product as compare to that of the AAV vector.

2. **Route of administration:** The vectors are administered together ***locally*** into the muscle or orthotropically into the diseased sites.

3. **Composition of the cocktail vectors:** In the immunocompetent rats, a therapeutic dose of AAV-BMP2 (5×10^{11} v.p.) and a non-cytotoxic dose of Adv-BMP2 (5×10^{8} v.p.) should be combined in order to achieve maximal enhancement of new bone formation.

Importantly, the cocktail vector system of the present invention can be optimized or modified, based on the information provided in the present disclosure, to carry any therapeutically effective gene product for treating many diseases or disorders.

In other words, It would be within the ability of one with ordinary skill in the art to replace the BMP-encoding nucleotide sequence in the cocktail vectors of the present invention with that encoding other therapeutic gene product and to locally treat various

conditions in a subject. Accordingly, Applicants believe no undue experimentation is necessary to practice the invention as recited in the amended claims.

In conclusion, Applicants believe that claims 13-16, 23-26, 29 and 30 as amended are fully enabling and, therefore, respectfully request that the rejection of these claims under 35 U.S.C. § 112, first paragraph, be withdrawn.

New Claims 31-48

New claims 31-48 are herein added.

Claims 31 and 32 depend from claim 13 or 23 and claim 33 depend from claim 23. Support can be found, for example, at page 5, lines 8-10; at page 4, lines 29-31; and at page 7, lines 7-16. Claims 34-48 are directed to methods for expressing BMP for new formation. Support for the claims can be additionally found, for example, at page 24, lines 21-29; at page 25, lines 6-10 and at page 41, lines 4-6. No new matter has been introduced.

Applicants believe the new claims are also fully enabling for the same reasons discussed for claims 13-16, 23-26, 29 and 30 in the previous section.

Consideration of new claims in addition to claims 13-16, 23-26, 29 and 30 is respectfully requested.

No fees, other than an extension fee is believed to be due for this submission. Should any fee(s) be required, please charge such fees to Deposit Account No. 50-2215.

Dated: June 30, 2006

Respectfully submitted,

By Edward A. Meilman

Edward A. Meilman
Registration No.: 24,735
DICKSTEIN SHAPIRO MORIN & OSHINSKY LLP
1177 Avenue of the Americas
New York, New York 10036-2714
212-277-6520
Attorney for Applicant

IY/CEM/mgs

Attachments: 1. Black/White replacement drawings (Figures 1A; 1B; 2; 4; 5A-5B; 6; 7A-7F; 10A-10C; 11; and 12;

2. Annotated version of Fig. 1A;

3. "Petition for Acceptance of Color Photographs" with three (3) sets of color drawings (Figures 3A-3B; 8A-8F; 9A-9F; and 13); and

4. "Submission of Substitute Sequence Listing and Statement in Accordance with 37 C.F.R. §§ 1.821-1.825" with Substitute Sequence Listing in paper form and CFR.